The Pineal lipid content in rat and the involvement of the epiphysis cerebri in the hypophyseo-gonadal interrelation

Zweens, Jacobus

SUMMARY

This investigation is concerned with certain aspects of the function of the pineal organ.

In *chapter I* a short historical survey is given together with a statement of the problem. Since the discovery of the pineal organ about 2000 years ago many speculations have been made as to its function. During 19 centuries the problem was dealt with in a rather philosophical way. During the last 60 years pineal function has been extensively studied in more exact and experimental ways. Amongst the various physiological roles attributed to the pineal organ, its influence on the hypophyseal-gonadal axis comes foremost. The possible repercussion of functional changes in the gonads or the adenohypophysis on the histological picture of the pineal organ has received but very little attention. Therefore this way of approaching the problem of pineal function was chosen in the present investigation in the hope that it would throw new light upon pineal function.

In *chapter II* a short description is given of the ontogenetic development and the morphology of the rat pineal organ. Furthermore, the literature concerning the histological changes in the pineal organ occurring with functional changes in the gonads is dealt with in this chapter. These data appear to be rather confusing and contradictory. Therefore, they do not permit to draw any conclusion as to the function of the pineal organ in mammals, the more so as the number of experimental animals used in these studies was - with a single exception - very small or even not mentioned at all. In the same chapter an orientating investigation concerning the histological picture of the pineal organ during different phases of the oestrous cycle is dealt with. It appeared that the amount of material which could be stained with the periodic acid SCHIFF technique (p.a.S.-positive material) was greater in di-oestrus than in pro-oestrus. Moreover, the pineal lipid content, demonstrable with Sudan black B

in propylene glycol after previous controlled chromation according to ELFTMAN, appeared to fluctuate during the oestrous cycle. During pro-oestrus the pineal lipid content is lowest. After ovulation it increases reaching its maximum at di-oestrus (fig. 1). No fluctuation in the lipid content of the outer border of the cerebellar cortex could be observed during the oestrous cycle. By this finding the specificity of the aforementioned fluctuation for the pineal organ is rendered very probable. After obtaining these results the question arose as to the cause and significance of the fluctuation in both pineal lipid content and p.a.S.-positive material. As the pineal lipid content is easier to quantify than the amount of p.a.S.-positive material, in the following experiments attention was mainly focused upon the pineal lipids.

In *chapter III* the colouring and measuring methods used in the determination of the pineal lipid content are discussed. The controlled chromation method following ELFTMAN (1954) was applied as a fixation technique for the pineal lipid material. From both literature data and own experimental data to be published elsewhere it was obvious that the total amount of phospholipid material and only part of the triglyceride material was preserved by this procedure. It was concluded therefore that the controlled chromation method is not specific for the demonstration of phospholipids, though the sensitivity of phospholipids for the chromation process is greatest. After fixation the lipid material was coloured with Sudan black B in propylene glycol. The use of propylene glycol as solvent for Sudan black B offers the advantage of a stable solution. Moreover propylene glycol is unable to dissolve lipids out of the tissues. Sudan black B shows a special tendency to be taken up by phospholipids. By this feature the phospholipid detection in tissues is rendered more easy. It was stated that both fixation and colouring method cooperate in demonstrating lipid material in general with a special affinity, however, for phospholipids. Furthermore, the histochemical picture of the pineal organ obtained after this fixation and colouring procedure was described. In the same chapter a short description is given of the method for determination of the pineal lipid content. It is stated that the values for the pineal lipid content obtained by the applied measuring method have to be considered semiquantitative.

In *chapter IV* the influence of ovariectomy and administration of

ovarian hormones on the pineal lipid content is dealt with. For the study of the pineal lipid content after ovariectomy, the experimental animals were ovariectomized when either 4 or 8 weeks old. They were sacrificed at different time intervals until 52 days after ovariectomy. From the values obtained for the pineal lipid content in the control animals it appeared that this content gradually decreases with increasing age, at least during the period of life investigated, *i.e.* from 4 to 15.5 weeks or otherwise said from immaturity to full maturity. Possibly, the fall of the pineal lipid content with increasing age must be interpreted as due to a general decrease in pineal metabolism. It also appeared that ovariectomy at the age of both 4 and 8 weeks is followed within 3 days by a steep rise of the pineal lipid content (figs. 3 and 4). The increase is even more pronounced following ovariectomy at the age of 8 weeks than it is after ovariectomy at the age of 4 weeks. In the pineal organs from animals, sacrificed 3 or more weeks after ovariectomy, areas varying in diameter from 100-500 μ can be found which are totally devoid of lipid material.

The influence of the administration of ovarian hormones on the pineal lipid content was studied in rats ovariectomized when 9 weeks old. The experimental animals were daily treated with either oestradiol benzoate or progesterone and sacrificed at different time intervals after ovariectomy. It appeared that daily administration of 10 mg progesterone was neither able to prevent the post-ovariectomy rise of the pineal lipid content (fig. 7), nor did it inhibit the development of the areas devoid of lipid material as seen in animals that were ovariectomized only. On the other hand, daily administration of 10 γ oestradiol appeared to be capable of annihilating the post-ovariectomy rise of the level of the pineal lipid content (fig. 6), the lipid level even dropping below the normal one. Moreover, daily treatment with oestradiol prevented the development of the lipidless areas which otherwise would have resulted from ovariectomy. These results strongly suggested that the pineal lipid content is influenced by hormone imbalances of the hypophyseal-gonadal axis.

In *chapter V* it is explained that on the base of these results two hypotheses can be advanced:

1. The pineal lipid content is influenced by the amount of circulating oestrogens.
2. The pineal lipid content is influenced by the serum level of gonadotropic hormones.

In the light of the first assumption, the results can be interpreted as follows. After ovariectomy the level of circulating oestrogens decreases sharply which might give rise to an increase of the pineal lipid content. Oestradiol administration in turn will result in a decrease of the pineal lipid content. Progesterone administration does not influence the serum level of oestrogens and therefore, does not alter the pineal lipid content. In this way, the experimental data fit in well with the assumption of an inverse relationship between both the amount of circulating oestrogens and the pineal lipid content.

In favour of the second assumption the results can be presented in the following way. It is a well-known fact that the serum level of gonadotropic hormones rises after ovariectomy. This rise might cause an increase of the pineal lipid content. It is also known that oestradiol administration diminishes the amount of circulating gonadotropic hormones and this in turn will bring about a decrease of the pineal lipid content. As progesterone administration in the doses used in this investigation does not affect the level of circulating gonadotropins, the pineal lipid content did not change after progesterone administration. Thus, the experimental data agree also very well with the assumption of a direct relationship between the pineal lipid content and the serum gonadotropin level.

The study of the influence of hypophysectomy on the pineal lipid content will reveal which of both hypotheses has to be rejected. For hypophysectomy diminishes the serum oestrogen level and, according to the assumption of an inverse relationship between oestrogens and pineal lipids, this ought to cause a rise in the pineal lipid content. However, according to the assumption of a direct relationship between pineal lipids and serum gonadotropins, the dissappearance of serum gonadotropins brought about by hypophysectomy ought to diminish the pineal lipid content.

Therefore, the influence of hypophysectomy and administration of gonadotropic hormones on the pineal lipid content is dealt with. The influence of hypophysectomy on the pineal lipid content was studied in rats hypophysectomized when 7 weeks old and sacrificed at regular time intervals after operation. It was demonstrated that hypophysectomy lowers the pineal lipid content to a level well below the normal one (fig. 9). This result strongly supports the hypothesis

of a direct relationship between the pineal lipids and the serum gonadotropin level and rules out the other hypothesis mentioned.

The validity of the hypothesis concerning the direct relationship between the pineal lipids and the serum gonadotropins has subsequently been tested. It was studied to which extent the administration of gonadotropic hormones could restore the normal level of the pineal lipid content in hypophysectomized animals. Female rats were hypophysectomized when 7 weeks old. The animals were daily treated with either pregnant mare serum gonadotropin (PMS) or human chorionic gonadotropin (HCG) and sacrificed at regular time intervals after operation. PMS-treatment appeared to restore the pineal lipid content in hypophysectomized animals to approximately normal values (fig. 12). Administration of HCG to hypophysectomized animals, however, raised their pineal lipid content to a level well above the normal one (figs 13 and 14). The gonadotropins used in this experiment were dissolved in saline, the ovarian hormones used in the previous experiment, however, in oil. It was demonstrated that neither sham-operation nor the administration of saline or oil did affect the pineal lipid content (fig. 11). From all experiments mentioned it was concluded that the pineal lipid content is influenced by the serum gonadotropin level. Ovarian hormones play no part in the interaction between the circulating gonadotropins and the pineal lipid content so far as the amount of lipid material is concerned. This was demonstrated by the fact that the rise of the pineal lipid content in hypophysectomized animals following treatment with PMS is not affected by ovariectomy. In these animals, however, ovariectomy gives also rise to the develpment of lipidless areas, similar to those seen in rats which are ovariectomized only.

The pineal organ is innervated by orthosympathetic fibres originating in the superior cervical ganglia of the orthosympathetic trunc. In *chapter VI* a study is made of the involvement of the autonomic nervous system in the interaction between pineal lipids and circulating gonadotropins. The pineal lipid content of animals in which the superior cervical ganglia were removed was compared to that of intact control animals. Moreover, the effect of PMS-administration was studied in both ganglionectomized and intact control rats. For this experiment immature females were used in

order to prevent a possible disturbance of the results by the oestrous cycle. The animals were kept in total darkness during the course of the experiment as, in this way, the decrease in the amount of light transmitted to the retina as a consequence of ganglionectomy was eliminated. No influence of ganglionectomy could be observed in this experiment. It was, therefore, concluded that in rat no argument is found for any regulatory involvement of the peripheral part of the autonomic nervous system in the interaction between the pineal lipid content and the serum gonadotropin level.

In *chapter VII* the involvement of circulating oestrogens in the distribution of the lipid material throughout the pineal organ is dealt with. It was stated before that in 3 groups of animals (ovariectomized, ovariectomized progesterone-treated and hypophysectomized ovariectomized PMS-treated) areas develop in the pineal organ which are totally devoid of lipid material. These groups of animals distinguish themselves from the other groups by an apparent lack of circulating oestrogens while circulating gonadotropins are present. The ovariectomized animals were chosen as being representative for the groups in which the lipidless areas occur and in these the general histological picture of the pineal organ, that of the lipidless areas in particular, was studied. Subsequent to controlled chromation, adjacent tissue sections were stained following the p.a.S. technique, the propylene glycol Sudan black B method and the MASSON-GOLDNER-WEIGERT technique respectively. The areas corresponding to the lipidless areas in the Sudan black B coloured material did not reveal any particular feature. Neither with the p.a.S. technique nor with the method following MASSON-GOLDNER-WEIGERT any histological difference could be observed between these areas on one hand and the surrounding pineal tissue or the pineal organs of control rats on the other. Concerning the cause of the occurrence of the lipidless areas different possible explanations were presented and tested as to their soundness. For the moment the areas devoid of lipid material are best explained by considering them to be the outcome of local depletion processes occasioned by the special hormonal condition in these animals. They suggest that apart from increasing the total pineal lipid content, the serum gonadotropins give also rise to local depletion of pineal lipids.

Against this effect the pineal organ is protected by the presence of a sufficiently high level of serum oestrogens.

Chapter VIII presents a general discussion which covers all results obtained by the present investigation. Up to now, a correlation between the pineal lipid content and the serum gonadotropin level has not been described in the literature concerning pineal function.

Recently, PANAGIOTIS and HUNGERFORD associated the pineal lipid content, detectable with oil red O, with changes in the body sodium and water balance. In a first subdivision of this chapter is dwelt upon the question whether their hypothesis offers an explanation for the results and conclusion to which our investigation has led. However, their results are not simply comparable with those described in the present investigation. Moreover, the literature data concerning the influence of the pineal organ on sodium and potassium balance, aldosterone production and the microscopic morphology are not conclusive at all. For the present, this renders the conception of PANAGIOTIS and HUNGERFORD very questionable. Furthermore, it does not offer a satisfactory explanation for our results. For these reasons it is very improbable that the changes in the pineal lipid content, described in the present investigation, are brought about by changes in the body sodium or serum aldosterone and angiotensin level or that they should be considered the outcome of fluctuations in pineal activity concerning the hormonal sodium regulation.

The fluctuations in the pineal lipid content being brought about by changes in the serum gonadotropin level, the question arises which of both hormones, either luteinizing hormone (LH) or follicle stimulating hormone (FSH), must be held responsible for affecting the pineal lipid content. In the second subdivision of this chapter it is concluded that, an influence of FSH being uncertain as yet, the effect of LH on the pineal lipid content is demonstrated convincingly.

Once more the problem of oestrogen involvement in the distribution of the lipid material throughout the pineal organ is discussed. For the moment, it is not possible to explain the occurrence of lipidless areas in the pineals of animals in which circulating gonadotropins are not counterbalanced by oestrogens.

Subsequently the introductory investigation, reported in chapter

II, is taken into consideration. The serum level of both FSH and LH are reported to fluctuate during the oestrous cycle. It is not possible as yet to correlate the cyclic fluctuation of the pineal lipid content exactly with any of the reported cyclic fluctuations in the serum LH or FSH level. However, the cyclic fluctuations in the pineal lipid content are best explained by assuming them to be caused by cyclic changes in the serum gonadotropin level.

Finally, the problem as to the functional significance of the serum gonadotropin induced changes in the pineal lipid content is discussed. On the base of recent literature and own experimental data it is concluded that in the juvenile rat the pineal organ inhibits the gonadotropic function of the anterior pituitary. This inhibitory action is of a humoral nature. It may be due to a pineal secretory product either affecting the production and/or release of gonadotropins in the adenohypophysis directly or by the way of the hypothalamus. As the pineal organ is involved in the control of the pituitary, the existence of a feedback system between both organs is very probable. The main characteristic of a feedback system is that the organs involved influence each other by their secretion products. The results of the present investigation demonstrate that the serum gonadotropins indeed affect the pineal lipid content while, on the other hand, the pineal affects the production of gonadotropins. Thus, the main requirement for the postulation of a feedback mechanism between the pineal organ and the anterior pituitary has been fulfilled, *i.e.* the mutual coupling of both organs. If this feedback mechanism will work adequately the serum gonadotropins must stimulate the antigonadotropic activity of the pineal organ. By the present investigation it was shown that lasting changes in the serum gonadotropin level cause corresponding changes in the pineal lipid content. Thus, a high or low pineal activity is attended with respectively a raised or lowered pineal lipid content. It was, therefore, concluded that the pineal lipid content can be used as a parameter for the antigonadotropic activity of the pineal organ.

SAMENVATTING

In dit onderzoek worden verschillende aspecten van de functie van de Epiphysis cerebri of pijnappelklier bestudeerd.

Hoofdstuk I: Na een kort historisch overzicht volgt een omschrijving van de probleemstelling van het onderzoek. Onder de talrijke functies welke aan de epiphyse worden toegeschreven is de invloed van dit orgaan op het hypophyse-gonadensysteem wel de belangrijkste. De mogelijke repercussie van veranderingen in de functietoestand van de gonaden of de adenohypophyse op het histologisch beeld van de epiphyse is echter nauwelijks bestudeerd. Daarom werd in dit onderzoek het probleem van de functie van de epiphyse op deze wijze benaderd in de hoop dat hierdoor het inzicht in de functie van de epiphyse eniszins verdiept zou worden.

Hoofdstuk II: Na een beknopte beschrijving van de ontogenetische ontwikkeling en de morphologie van de epiphyse van de rat, volgt een literatuuroverzicht. Hierna wordt een oriënterend onderzoek naar het histologisch beeld van de epiphyse in verschillende phasen van de ovariële cyclus gerefereerd. Zowel de hoeveelheid materiaal kleurbaar met de perjoodzuur-SCHIFF techniek, (p.a.S.-positief materiaal), als de hoeveelheid lipiden, aantoonbaar met Sudan black B na gecontroleerde chromering (ELFTMAN, 1954), bleken te variëren gedurende de ovariële cyclus. Tijdens de pro-oestrus is de hoeveelheid van beiden het geringst om na de ovulatie toe te nemen en het maximum te bereiken tijdens de di-oestrus (fig. 1). Door dit resultaat kwam de vraag naar voren naar de oorzaak en betekenis van deze fluctuatie. In de volgende proeven werd de aandacht vooral op de lipiden gericht, omdat deze betrekkelijk eenvoudig gequantificeerd konden worden.

Hoofdstuk III: Bespreking van de kleur- en meetmethode met behulp waarvan de lipiden in de epiphyse werden bepaald. Met name is aandacht besteed aan de histochemische aspecten van de gecontro-

leerde chromering (ELFTMAN, 1954) gevolgd door kleuring met Sudan black B.

Hoofdstuk IV: Beschrijving van de invloed van ovariectomie en toediening van ovariële hormonen op het lipidegehalte van de epiphyse. Allereerst bleek dat onder normale omstandigheden en na ovariectomie de hoeveelheid lipiden in de epiphyse daalde met het ouder worden van de dieren, althans tot het moment van volwassenheid. Mogelijk moet dit verklaard worden op grond van een daling in het metabolisme van de epiphyse. Na ovariectomie stijgt de hoeveelheid lipiden in de epiphyse snel (fig. 3 en 4). Vanaf 3 weken na ovariectomie ontwikkelen zich in de epiphyse gebieden waarin geen lipiden aantoonbaar zijn. Met behulp van dagelijkse progesterontoediening kon bij geovariectomeerde dieren noch de stijging van de hoeveelheid lipiden voorkomen worden (fig. 7), noch het ontstaan van de genoemde vetlege plekken. Door dagelijkse oestradioltoediening daarentegen kon bij geovariectomeerde dieren zowel de stijging van het lipidegehalte van de epiphyse voorkomen worden (fig. 6), als het ontstaan van vetlege plekken. Onder invloed van oestradiolbehandeling daalde het lipidegehalte zelfs tot beneden de normale waarde. Op grond van deze resultaten leek het waarschijnlijk dat het lipidegehalte van de epiphyse beïnvloed wordt door het hypophyse-gonadensysteem.

Hoofdstuk V: De voorgaande resultaten kunnen verklaard worden op grond van twee verschillende hypothesen:

1. Het lipidegehalte van de epiphyse wordt beïnvloed door de hoeveelheid circulerende oestrogenen.
2. Het lipidegehalte van de epiphyse wordt beïnvloed door de hoeveelheid circulerende gonadotrope hormonen.

Vervolgens wordt uiteengezet dat, indien de eerste hypothese juist is, het lipidegehalte van de epiphyse moet stijgen na hypophysectomie. Is daarentegen de tweede hypothese juist, dan zou het lipidegehalte van de epiphyse moeten dalen na hypophysectomie..

Uit een volgend experiment bleek dat hypophysectomie de hoeveelheid lipiden in de epiphyse doet dalen (fig. 9). Dit steunt de veronderstelling dat het lipidegehalte van de epiphyse wordt beïnvloed door de serum gonadotropinen. Hierna werd onderzocht in hoeverre toediening van gonadotrope hormonen aan gehypophysectomeerde dieren het lipidegehalte van de epiphyse weer deed stijgen.

Als gonadotropinepreparaten werden 'pregnant mare serum gonadotropin' (PMS) en 'human chrorionic gonadotropin' (HCG) gebruikt. Het bleek dat toediening van PMS de hoeveelheid lipiden in de epiphyse van gehypophysectomeerde dieren terugbracht tot ongeveer de normale waarde (fig. 12). Toediening van HCG in plaats van PMS deed echter het lipidgehalte van de epiphyse duidelijk tot boven de normale waarde stijgen (fig. 13 en 14). Ovariumhormonen spelen geen rol in de beïnvloeding van de hoeveelheid lipiden in de epiphyse door de gonadotrope hormonen. Dit bleek duidelijk uit het feit dat de stijging van de hoeveelheid lipiden tengevolge van PMS-behandeling in de epiphyse van gehypophysectomeerde dieren niet werd beïnvloed door ovariectomie. Wel ontstonden bij deze dieren gebieden in de epiphyse waarin geen lipiden aantoonbaar waren.

Hoofdstuk VI: De epiphyse wordt orthosympatisch geïnnerveerd vanuit het ganglion cervicale superius van de truncus orthosympathicus. Deze innervatie behoeft niet intact te zijn voor de regulerende werking van de serum gonadotropinen ten opzichte van het epiphysaire lipidegehalte.

Hoofdstuk VII: Hier wordt ingegaan op de invloed van circulerende oestrogenen op de verdeling van het lipide materiaal in de epiphyse. De reeds genoemde vetlege gebieden in de epiphyse blijken alleen voor te komen bij dieren met een sterk verlaagde serum oestrogenen spiegel naast voldoende circulerende gonadotropinen. Dit gebrek aan circulerende oestrogenen bij een voldoende hoeveelheid serum gonadotropinen, leidt tot locale lipide depleties binnen de epiphyse. Een andere verklaring voor het voorkomen van deze vetlege gebieden lijkt voorlopig onmogelijk. Naast de algemene verhoging van de hoeveelheid lipiden in de epiphyse zouden de serum gonadotropinen dan locaal vetdepleties in de epiphyse moeten bewerkstelligen. Hiertegen zou de epiphyse op zijn beurt weer beschermd worden door de aanwezigheid van voldoende oestrogenen in het serum.

Hoofdstuk VIII: Algemene discussie. In de literatuur over de functie van de epiphyse is een correlatie tussen de serum gonadotropinen spiegel en de hoeveelheid lipiden in de epiphyse niet eerder beschreven. Wel menen PANAGIOTIS en HUNGERFORD onlangs een samenhang gevonden te hebben tussen enerzijds de hoeveelheid epiphysaire lipiden, aantoonbaar met oil red O, en anderzijds ver-

anderingen in de natrium- en vochtbalans van het lichaam. Na een bespreking van hierop betrekking hebbende literatuur wordt uiteengezet dat onze resultaten niet verklaard kunnen worden op grond van veranderingen in de natrium- en vochtbalans van het lichaam.

Vervolgens wordt uiteengezet dat de invloed van luteiniserend hormoon (LH) op het lipidegehalte van de epiphyse duidelijk is aangetoond, terwijl een dergelijk effect van follikel-stimulerend hormoon (FSH) vooralsnog niet bevestigd of uitgesloten kan worden. De fluctuaties van de hoeveelheid lipiden in de epiphyse tijdens de ovariële cyclus moeten opgevat worden als een weerspiegeling van veranderingen van de serum gonadotropinenspiegel tijdens de cyclus.

Als sluitstuk van de discussie volgt een bespreking van de betekenis welke gehecht moet worden aan de fluctuatie van het lipidegehalte van de epiphyse onder invloed van de serum gonadotropinen. Op grond van recente literatuur en eigen experimentele gegevens wordt geconcludeerd dat in onvolwassen ratten de gonadotrope functie van de hypophyse wordt geremd door de epiphyse. Aangezien de epiphyse betrokken is bij de regulatie van de hypophysefunctie, zal tussen beide organen naar alle waarschijnlijkheid een 'feedbackmechanisme' bestaan. Beide organen beïnvloeden elkaar via hun secretieproducten: het belangrijkste kenmerk van een 'feedbackmechanisme'. Indien dit 'feedbackmechanisme' adaequaat werkt moeten de serumgonadotropinen de antigonadotrope activiteit van de epiphyse stimuleren. In dit onderzoek is gebleken dat langdurige veranderingen in de serum gonadotropinenspiegel overeenkomstige veranderingen in het lipidgehalte van de epiphyse veroorzaakt. Dit impliceert dat een verhoogde of verlaagde antigonadotrope activiteit van de epiphyse gepaard gaat met respectievelijk een verhoogd of verlaagd lipidegehalte van de epiphyse. Op grond hiervan werd geconcludeerd dat het lipidegehalte van de epiphyse gebruikt kan worden als parameter voor de antigonadotrope functie van dit orgaan .